20 cc. of concentrated hydrochloric acid in Babcock cream bottles. The bottles were immersed in boiling water for 5 minutes, after which the fat was separated by the Babcock centrifuge, filtered, and compared with the fat obtained upon the same cream as in the regular Babcock method. The results are shown in Table II.

Table II

Fat obtained by	Babcock method	Hydrochloric acid method
Refractometer reading, 40° C.	41.8	41.8
Koettstorfer number	227.0	228.0
Reichert-Meissl number	28.3	29.0

Hydrochloric acid, therefore, was not retained by the fat to any appreciable extent, 5 grams having an acidity equivalent to only 0.5 cc. of 0.1 *N* NaOH. The Koettstorfer and Reichert-Meissl figures were practically identical with those obtained when using sulfuric acid for the decomposition.

Table III—Results from Treatments of Pure Butter

Sample No.	Determination	Untreated	Treated as in Babcock method	Treated as in HCl method	Treated as in Acetic Acid Method: Not Heated	Heated at 90–95° C.: 1/4 hour	1/2 hour	1 hour	2 hours
1	Refractometer	40.7	40.6	40.6	36.9	38.7	39.7	40.6	40.6
	Koettstorfer	226.0	..	227.0	..	..	..	226.0	..
	Reichert-Meissl	29.7	..	29.4	39.5	..	..	29.3	..
2	Refractometer	42.0	42.0	42.0	38.8	40.2	41.0	42.0	..
	Reichert-Meissl	...	..	...	41.6	..	..	28.2	..
3	Refractometer	42.9	43.0	42.9	39.8	41.6	42.8	42.9	..

Experiment III—In order to further show that acetic acid is the only acid retained to a sufficient extent to affect the refractive index and other constants of butter fat, three samples of pure butter were melted and the filtered fat treated by shaking in a separatory funnel with such volumes of water and acid as approach the proportions of each present in the analysis as ordinarily carried on. The quantities used were as follows:

a—*Babcock Method:* 15 cc. melted fat, 30 cc. water, 40 cc. concentrated sulfuric acid.

b—*Hydrochloric Acid Method:* 15 cc. melted fat, 90 cc. hot water, 100 cc. concentrated hydrochloric acid.

c—*Acetic Sulfuric Acid Method:* 15 cc. melted fat, 30 cc. water, 100 cc. glacial acetic acid, 50 cc. concentrated sulfuric acid.

After shaking thoroughly for 3 minutes, the fat was allowed to come to the top, filtered, and cooled in the refrigerator. The refractometer readings were then taken upon all, and the Koettstorfer and Reichert-Meissl numbers upon a few, of the samples. The results were compared with the constants obtained upon the untreated fats, as shown in Table III. The refractometer readings were also taken upon the fat treated with acetic acid, after standing for certain time intervals in an air oven at 90–95° C.

This table indicates that abnormal results are obtained only in the case of fats separated in the presence of acetic acid. Such results are due to acetic acid being mechanically held by the fat. There is no acetylization. The fat obtained by the acetic acid method after thorough washing to remove acidity and treating as in the determination of the acetyl value, but without acetylating with acetic anhydride, gives results which do not differ from those upon the untreated fat.

The refraction reading and other constants are the most erratic when the greatest amount of acetic acid is present, but approach nearer to the correct results as the acid is gradually volatilized. One hour's heating at 90–95° C. is necessary to drive off all of the acetic acid, after which normal figures for all of the determinations may be obtained.

CONCLUSIONS

I—Fats separated from dairy products by decomposition with sulfuric or hydrochloric acid will give the correct results for all of the usual determinations without any further treatment.

II—Fats separated in the presence of acetic acid will retain this acid and cause erroneous results. The acid is retained mechanically and must be entirely removed by drying for one hour at 90–95° C. before proceeding to determine the constants.

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STUDIES IN SYNTHETIC DRUG ANALYSIS
IV—ESTIMATION OF PHENACETIN AND SALOL IN ADMIXTURE

By W. O. Emery, G. C. Spencer and C. C. LeFebvre

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INTRODUCTION

In the course of certain analytical work on medicinal tablets, we were considerably handicapped for a time through lack of a quantitative method for estimating phenacetin and salol in admixture. Accordingly, efforts were directed first to their separation and second to their individual determination. It was found that a satisfactory separation could be effected by treatment either with dilute sulfuric acid or with sodium hydroxide of definite concentration, followed by extraction with chloroform.

The separation proper, therefore, follows two distinct lines of procedure, involving on the one hand the acid hydrolysis of phenacetin, on the other the alkaline hydrolysis of salol. In the first instance, the direct estimation of phenacetin only is contemplated, the salol being determined by difference in all cases where the combined weight of both constituents is known or can be conveniently ascertained. In this procedure, the phenacetin is temporarily converted into phenetidine sulfate and acetic acid, while in the case of salol partial volatilization results during the process of digestion. The phenetidine salt, by virtue of its insolubility in chloroform, is readily freed from further contaminating salol by treatment with that solvent, then by means of acetic anhydride and sodium bicarbonate reconverted into phenacetin, the latter being subsequently isolated and weighed.

In the case of alkaline hydrolysis, the salol is first converted into sodium salicylate and phenolate, from which the phenacetin is easily separated and subsequently recovered by extraction with chloroform. The salol is thereupon treated in rotation with standard bromine solution in excess, hydrochloric acid, potassium iodide and sodium thiosulfate. The principal reactions involved in the process and which serve as the basis for the calculation of results are:

$$HOC_6H_4CO.OC_6H_5 + 3NaOH = NaOC_6H_4CO_2Na + C_6H_5ONa + 2H_2O$$

$$C_6H_5OH + 4Br_2 = C_6H_2Br_3OBr + 4HBr$$

$$HOC_6H_4CO.OH + 4Br_2 = C_6H_2Br_3OBr + 4HBr + CO_2$$

$$C_6H_2Br_3OBr + 2HI = C_6H_2Br_3OH + HBr + I_2$$

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6$$

from which it follows that 12 atoms of bromine are expended on every atom of salol and that titration of the iodine liberated by the unexpended bromine affords sufficient data for the estimation of salol.

FIRST METHOD—ACID HYDROLYSIS

PHENACETIN—If the sample is in pill or tablet form, ascertain the weight of 20 or more, reduce to a fine powder and transfer to a small tube or specimen bottle which can be kept tightly closed with a cork or glass stopper. Weigh out on a small (5.5 cm.) tared filter an amount of the sample equal to, or a convenient multiple of, the average weight of such pill or tablet, wash with successive small portions of chloroform, in quantity about 40 cc., sufficient at least to insure the complete extraction of all the phenacetin and salol present in the mixture. Collect the solvent in a tared 100 cc. beaker, evaporating by means of a blast to apparent dryness. During this operation, the beaker may, with advantage, be allowed to stand on a warm plate (50–60°) without danger of undue loss of solid substance. Permit the beaker to remain 24 hours in the open at the ordinary temperature, weigh several times until practically constant, then transfer the crystalline residue, by dissolving in and washing with sufficient chloroform, to a 50 cc. lipped Erlenmeyer, evaporate solvent by means of a blast and gentle heat, add 10 cc. dilute sulfuric acid (1 : 10) and digest at full steam-bath heat until the liquid is reduced one-half. Add 10 cc. of water, continue the digestion as before, add a second 10 cc. of water and evaporate to 5 cc. Transfer the residual liquid by pouring and washing with about 20 cc. of water to a small separatory funnel of the Squibb type and extract in rotation with 15, 10 and 5 cc. chloroform, washing each portion as obtained with 5 cc. water in a second separator, in order to recover traces of phenetidine sulfate possibly taken up by the chloroform, finally rejecting the latter, since it contains all the salol not previously eliminated during the process of digestion.

To the aqueous acid solution of phenetidine sulfate in the first separatory funnel, into which also the wash water from the second separator has been drawn, add sodium bicarbonate in small portions until an excess of this reagent persists at the bottom of separator after complete neutralization of the sulfuric acid. Now add 25 cc. chloroform and for every 100 mg. acetphenetidine or phenacetin known or believed to be present 5 drops of acetic anhydride, shake for some time vigorously, then pass the nearly clarified solvent into a second separator containing 5 cc. water, shake and receive the chloroform in a 200 cc. Erlenmeyer, first passing it through a small (5.5 cm.) dry filter. Distil at gentle heat about 20 cc. returning the latter to the first separator. Augment with 5 cc. fresh chloroform and repeat the extraction, washing and filtering as before, then collecting the solvent in the Erlenmeyer and again distilling until 25 cc. chloroform are obtained. With this portion make a third and final extraction, repeating the operations of washing, filtering and distilling to the point where about 5 cc. chloroform remain in the Erlenmeyer. Transfer this chloroformic residue by pouring and washing with sufficient fresh chloroform to a tared 50 cc. beaker or crystallizing dish, evaporate on a steam or vapor bath to apparent dryness, finally removing any pronounced excess of acetic anhydride by repeated addition of 1 cc. portions of fresh chloroform (to which a drop of alcohol has been added) and subsequent evaporation. Allow the nearly colorless crystalline residue of acetphenetidine or phenacetin to stand some time in the open, or better in a vacuum desiccator over lime, in order to eliminate the last traces of acetic anhydride. Weigh at intervals until constant.

SALOL—To ascertain the quantity of salol present in the sample, subtract the weight of phenacetin as found above from the combined weight of the two ingredients determined in the preliminary separation.

SECOND METHOD—ALKALINE HYDROLYSIS

PHENACETIN—On a small tared filter weigh out an amount of the sample containing not more than 100 mg. salol, exhaust with chloroform as in acid hydrolysis, collecting the solvent in a small lipped Erlenmeyer. Evaporate the chloroform by means of a blast and without applying heat. Add 10 cc. 2.5 per cent sodium hydroxide solution and heat 5 minutes on a steam or vapor bath at the temperature of boiling water. At the end of this period remove from bath and cool quickly to room temperature in running water in order to reduce to a minimum any tendency of the phenacetin to undergo partial hydrolysis. Transfer the liquid to a separatory funnel by pouring and washing with a minimum of water, finally rinsing out the flask with the first 20 cc. portion of chloroform used in extraction. Extract the aqueous alkaline solution with three 20 cc. portions of chloroform, washing each portion as obtained consecutively in a second separator with 5 cc. water, prior to passing the solvent through a small, dry filter into a 200 cc. Erlenmeyer, from which the combined chloroformic portions are distilled down to a residue of about 5 cc. Transfer the latter by pouring and washing with sufficient fresh chloroform to a small tared beaker or crystallizing dish, evaporate the solvent on the steam bath by the aid of a blast, cool and weigh the phenacetin at intervals until constant.

SALOL—Transfer both aqueous alkaline solutions from the separatory funnels to a suitable (500 cc.) glass-stoppered bottle, dilute with water to about 200 cc., run in from a burette an excess (about 45 cc.) of $N/7$ potassium bromide bromate, follow with 10 cc. concentrated hydrochloric acid, close flask and shake 1 minute, then at intervals over a period of one-half hour. At the end of this time add 10 cc. 15 per cent potassium iodide solution, agitating the closed flask at intervals for 15 minutes. Titrate the free iodine with standard thiosulfate (preferably $N/7$), previously adjusted to the standard bromine solution, 1 cc. of which is equivalent to 0.002548 g. salol. From the number of cubic centimeters standard bromine solution expended, calculate the salol on the basis of 12 atoms of bromine to 1 molecule of salol.

COMMENTS AND SUGGESTIONS

Tared filters for use in this and similar work may be conveniently prepared as follows: Fold, adjust in a small short-stemmed funnel, moisten and press carefully so that the entire rim of filter fits snugly to the glass. After drying, keep in balance case or other suitable dust-proof container, and when needed weigh on a small metal support or glass tripod. It is self-evident that all weighings with tared filters should be made as nearly as possible under comparable conditions. If, for example, the first weight is taken under a hygrometric reading of 50 per cent, the second weighing should be made under approximately similar conditions. This is indispensable when it is desired to estimate the quantity of chloroform-insoluble residue in phenacetin-salol tablets or other preparations likely to contain sodium bicarbonate, sugar, starch, talc or other more or less inert excipients. Experience has shown, however, that a variation in humidity up to 5 per cent at the time of the two weighings would not be productive of material error.

Before carrying out extractions in separatory funnels or introducing liquids therein, the valves should first be "locked" with a drop of water, otherwise losses from capillarity may result.

All distillations of chloroform should be carried out over a wire gauze or asbestos provided with a small opening and heated by a very small flame, the flask being connected with the condenser by means of a small spray trap, if available, similar to the type used in Kjeldahl work.

Phenacetin, being only moderately soluble in hot aqueous media, is accordingly somewhat difficult of hydrolysis. In order, therefore, to insure its complete conversion to phenetidine sulfate, special attention should be directed to the end that any visible particles of crystalline substance on the sides of flask during the early part of digestion should be gradually brought into solution by gently rotating the flask and, if necessary, by the occasional addition of a few drops of chloroform.

In order to facilitate the work and minimize errors due to recharging burettes, $N/7$ solutions are advocated in order that the volume of the thiosulfate required may not exceed the capacity of a 50 cc. burette. The thiosulfate solution is standardized against pure iodine. The strength of the bromine solution may also be determined by the aid of pure acetanilide.[1]

EXPERIMENTAL

The tabulated data given below are representative of results obtained by the authors in operations carried out on control and commercial mixtures substantially in accordance with the methods outlined above. A slight variation from the prescribed procedure should, however, be noted in the second series,

ACID HYDROLYSIS

SERIES 1

Phenacetin	Salol	Phenacetin recovery		Salol recovery	
Gram	Gram	Gram	Per cent	Gram	Per cent
0.1028	0.1159	0.1028	100.00	0.1159	100.00
0.1021	0.0996	0.1021	100.00	0.0996	100.00
0.1006	0.1048	0.1009	100.39	0.1045	99.71
0.1015	0.1015	0.1010	99.50	0.1020	100.49
0.1010	0.1044	0.1008	99.80	0.1046	100.19
0.1042	0.1004	0.1043	100.09	0.1003	99.92
0.1015	0.1001	0.1016	100.09	0.1000	99.90
0.1069	0.1186	0.1071	100.18	0.1184	99.83
0.1010	0.1174	0.1009	99.89	0.1175	100.09
0.0996	0.1014	0.0998	100.20	0.1012	99.79
0.1103	0.1036	0.1101	99.82	0.1038	100.19
0.2000	0.2000	0.1999	99.95	0.2001	100.05
0.1000	0.2000	0.0995	99.50	0.2005	100.25
0.2000	0.1000	0.2004	100.20	0.0996	99.60

SERIES 5

SAMPLE(*a*)	Phenacetin + Salol recovery	Phenacetin recovery		Salol recovery	
Gram	Gram	Gram	Grains	Gram	Grains
0.4316	0.3256	0.1621	2.50	0.1635	2.52
0.4316	0.3253	0.1622	2.50	0.1631	2.52
0.4316	0.3257	0.1616	2.49	0.1641	2.53
0.4316	0.3273	0.1626	2.51	0.1647	2.54
0.4316	0.3276	0.1607	2.48	0.1669	2.58
0.4316	0.3268	0.1611	2.49	0.1657	2.56
0.4316	0.3268	0.1637	2.53	0.1631	2.52

ALKALINE HYDROLYSIS

SERIES 2

PHENACETIN	SALOL	Phenacetin recovery		$N/7$ Koppeschaar solution	Salol recovery	
Gram	Gram	Gram	Per cent	Cc. expended	Gram	Per cent
....	0.0892	....	....	33.99	0.0882	98.88
....	0.0895	....	....	35.06	0.0891	99.64
....	0.0900	....	....	35.17	0.0894	99.37
....	0.0903	....	....	35.20	0.0893	99.12
0.0125	0.0914	0.0123	98.40	35.27	0.0897	98.13
0.0141	0.0915	0.0142	100.70	35.54	0.0904	98.77
0.0516	0.0513	0.0516	100.00	20.39	0.0518	101.10
0.0516	0.0512	0.0511	99.04	20.59	0.0523	102.30
0.0503	0.0499	0.0509	101.20	19.92	0.0506	101.50
0.0504	0.0508	0.0513	101.70	20.35	0.0517	101.77
0.0495	0.0499	0.0486	98.18	19.69	0.0501	100.40
0.0522	0.0516	0.0503	97.32	20.52	0.0521	100.97
0.0891	0.0884	0.0867	97.30	34.92	0.0890	100.67
0.1020	0.1020	0.1002	98.22	39.37	0.1003	98.33
0.1000	0.1003	0.0983	98.30	38.82	0.0989	98.63
....	0.0880	....	....	34.67	0.0883	100.30
....	0.0896	....	....	35.23	0.0898	100.20
0.0897	0.0892	0.0880	98.11	35.47	0.0904	101.30
....	0.1020	....	....	39.35	0.1002	98.32
0.0977	0.0999	0.0974	99.70	38.63	0.0984	98.55
0.1005	0.1008	0.0999	99.42	39.71	0.1012	100.39
0.0989	0.1012	0.0982	99.28	40.11	0.1022	101.00
0.1011	0.1009	0.1000	98.91	40.00	0.1019	101.00
SERIES 3						
0.1006	0.1000	0.1002	99.60	39.48	0.1006	100.60
0.1039	0.1017	0.1011	97.31	40.00	0.1019	100.20
0.1015	0.1014	0.0987	97.25	39.69	0.1011	99.75
0.0995	0.1200	0.0981	98.60	47.11	0.1200	100.00
0.1012	0.1010	0.0995	98.33	39.64	0.1010	100.00
0.0982	0.0991	0.0966	98.37	38.76	0.0987	99.58
0.1023	0.1000	0.0998	97.57	39.12	0.0997	99.70
0.0996	0.1016	0.0972	97.59	39.86	0.1016	100.00
0.1037	0.0988	0.1013	97.68	38.78	0.0988	100.00
SERIES 4						
0.1032	0.1014	0.1035	100.20	39.60	0.1009	99.54
0.1043	0.0978	0.1045	100.20	38.22	0.0974	99.58
0.1003	0.1011	0.0990	98.70	39.83	0.1015	100.40
0.0911	0.1012	0.0894	98.13	39.93	0.1017	100.49
0.0723	0.1015	0.0710	98.20	40.08	0.1021	100.59
0.0569	0.1020	0.0569	100.00	39.87	0.1016	99.61
0.0799	0.1002	0.0787	98.49	39.47	0.1006	100.30
0.0553	0.1092	0.0550	99.45	42.71	0.1088	99.68

SERIES 6

SAMPLE(*a*)	Phenacetin recovery		$N/7$ Koppeschaar solution	Salol recovery	
Gram	Gram	Grains	Cc. expended	Gram	Grains
0.2700	0.1005	2.48	40.06	0.1022	2.52
0.2700	0.1002	2.47	40.15	0.1023	2.52
0.2700	0.1003	2.47	40.04	0.1021	2.52
0.2641	0.0982	2.48	39.08	0.0996	2.51
0.2641	0.0985	2.48	38.82	0.0989	2.49

(*a*) Average weight of single tablet 0.4316 g.

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[1] *Bull.* **132**, Bureau of Chemistry, U. S. Dept. Agr.; *Proc. A. O. A. C.*, p. 198, footnote *b*.

namely, that a 5 per cent sodium hydroxide solution was employed in effecting hydrolysis; likewise, in the fourth series, where two 30-cc. and one 20-cc. portions of chloroform were used in the recovery of phenacetin.

Series 5 and 6 involve a commercial preparation in tablet form alleged to contain 2.5 grains (0.1620 g.) each of phenacetin and salol.

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THE PRODUCTION, BOTANICAL COMPOSITION AND VOLATILE OIL STRENGTH OF AMERICAN WILD MUSTARD SEED

By A. L. Winton and J. H. Bornmann

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The term "Wild Mustard," although regarded by botanists as being synonymous with charlock (*Brassica arvensis*), is applied by the trade to variable mixtures of charlock with brown mustard (*Brassica juncea*). Charlock is one of the most troublesome weeds in the grain and flax fields of the Northwest. Notwithstanding the means employed for its eradication by both state authorities and farmers, many of the fields of the Dakotas, Minnesota and adjoining states are masses of yellow during the flowering season and the threshed grain and flaxseed are contaminated with wild mustard seed. Other plants belonging to the mustard family occur with the charlock but most of these, such as pepper grass (*Lepidium virginicum*), shepherd's purse (*Capsella bursa-pastoris*) and false flax (*Camelina sativa*), do not have globular seeds. The notable exception is brown mustard, the seeds of which resemble those of charlock in size and almost invariably occur in greater or less amount mixed with the latter in the so-called "Wild Mustard" separated at the elevators, flour mills, malt houses, seed warehouses, and linseed works.

The total production of so-called wild mustard is very large. Samples of wheat examined in a single day under the direction of Mr. G. H. Tunnell, Chief State Grain Inspector, Minneapolis, in the presence of one of the writers, contained as high as 2.15 per cent of wild mustard and much larger amounts were stated to be not uncommon. A sample representing the total screenings output of one of the large Minneapolis mills contained 11.04 per cent of wild mustard of which 10.74 per cent was charlock and 0.30 per cent brown mustard.

Assuming an average of only 0.1 per cent for the entire spring wheat crop of 1913, the grand total would be about 180 carloads of 40 tons each. This estimate, which is doubtless much too low for the wheat crop, does not include the wild mustard separated from barley, rye and linseed, and that produced in adjoining provinces of Canada.

A grain merchant thoroughly acquainted with the situation reported that upward of 100 carloads annually were separated from grain in Minneapolis alone and another trade authority stated that this estimate was much too low. Large quantities are said to be shipped to Europe.

SEPARATION OF WILD MUSTARD FROM GRAIN AND OTHER SEEDS

Screenings consisting of foreign seeds, chaff and other impurities are first separated into different grades by means of sieves and air currents. The grade containing the wild mustard seed includes other seeds, notably green fox tail (*Chaetochloa viridis*), and seed fragments of about the same size which are removed by ingenious machines, depending on the fact that these impurities do not roll with as great ease on an inclined surface as the round seed of wild mustard.

Two types of power separators, namely, the belt and the disk machines, are in common use and recently a spiral "extractor" containing no moving parts has been placed on the market.

THE BELT MACHINE consists of a broad belt running on two horizontal pulleys, one of which is higher than the other so that the upper surface of the belt forms an inclined plane moving slowly forward toward the higher pulley. A broad but thin stream of screenings is continually delivered on the belt from a hopper. The round seeds of the wild mustard roll down the belt into a suitable receptacle while the other seeds, because of their irregular form, do not roll but are carried by the belt in the opposite direction and discharged at the upper end.

THE DISK MACHINE employs several metal disks sloping slightly toward the center and revolving on the same vertical shaft. The screenings are delivered at a point near the edge of each disk. As the disk turns, the wild mustard seeds roll toward the center where they enter a chute, while the other seeds are carried around and are brushed off near the feeding point.

THE SPIRAL "EXTRACTOR" consists of a stationary spiral with several turns made up of three chutes into which the screenings are fed through a hopper. In their passage downward, the wild mustard seeds acquire sufficient velocity to jump outward into the middle and finally the outer chute from which in turn they pass into a hopper outlet, while the other seeds and impurities remain in either the middle or inner chute and emerge through two other outlets.

DISTINCTION BETWEEN CHARLOCK AND BROWN MUSTARD

The important distinctions between the two seeds which make up the wild mustard of commerce are as follows:

1—Brown mustard is an escaped plant which in Russia is cultivated for its seed; charlock is an introduced weed regarded everywhere as a pest.

2—Brown mustard is dark brown and under a lens shows distinct reticulations; charlock varies from dark brown to nearly black and under a lens is finely granular but not reticulated.

3—The hulls of brown mustard do not respond to the chloral hydrate test; the hulls of charlock gently heated with this reagent become carmine-red.[1]

4—Brown mustard, like black mustard (*Brassica nigra*), contains potassium myronate, which on treatment with water, owing to the action of the myrosin, splits up with the formation of volatile mustard oil;

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[1] Waage, *Ber. Pharm. Ges.*, **1893**, p. 153; Winton, *Orig. Com. 8th Intern. Cong. Appl. Chem.*, **26** (1912), 409.